Transmission of Ockelbo Virus by *Aedes cinereus*, *Ae. communis*, and *Ae. excrucians* (Diptera: Culicidae) Collected in an Enzootic Area in Central Sweden

MICHAEL J. TURELL,[1] JAN O. LUNDSTRÖM,[2,3] AND BO NIKLASSON[2,4]

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ABSTRACT Studies were conducted to determine the ability of *Aedes excrucians* (Walker), *Ae. cinereus* Meigen, and *Ae. communis* (De Geer) group mosquitoes, collected in an Ockelbo (OCK) virus enzootic area in central Sweden, to transmit this virus. All three species were highly susceptible to infection; at least 96% of the specimens of each species became infected after ingesting blood from a viremic chicken. Recovery of virus from the legs of all 61 of the *Ae. excrucians* and from 51% (24 of 47) and 75% (6 of 8) of the *Ae. cinereus* and *Ae. communis* tested, respectively, indicated that OCK virus readily disseminated from the midgut to the hemocoel in these species. Although none of the *Ae. communis* refed, *Ae. cinereus* and *Ae. excrucians* successfully transmitted OCK virus by bite. Because these *Aedes* species are attracted to avian and mammalian hosts and because OCK virus has been isolated from field-collected specimens in Sweden and the USSR, *Aedes* mosquitoes should be considered a possible link between human infections and the enzootic cycle involving birds and *Culex* and *Culiseta* mosquitoes.

KEY WORDS Insecta, *Aedes*, alphavirus, Ockelbo virus

OCKELBO (OCK) DISEASE and the clinically identical Pogosta disease and Karelian fever are characterized by fever, rash, and arthralgia. These diseases are endemic in central Sweden, Finland, and the Karelian region of the Soviet Union, respectively. Based on serological evidence, these diseases are caused by Sindbis-like viruses (Brummer-Korvenkontio & Kuusisto 1981, Skogh & Espmark 1982, Lvov et al. 1982). An alphavirus was isolated from *Culiseta* mosquitoes collected in the enzootic area of Sweden during the 1982 outbreak (Niklasson et al. 1984) and was shown serologically to be associated with OCK disease (Espmark & Niklasson 1984). This virus, designated OCK virus, serologically is closely related to, but distinguishable from, Sindbis virus.

Serological studies in birds and attempts to isolate virus from mosquitoes have indicated a natural enzootic cycle between passerine birds and either *Culex* or *Culiseta* mosquitoes (Francy et al. 1989). However, the mosquitoes implicated (*Culex pipiens* (L.), *Cx. torrentium* Martini, and *Culiseta morsitans* (Theobald)) are essentially avian feeders (Service 1971, Jaenson & Niklasson 1986); thus, they may not be responsible for viral transmission to man. In addition to the numerous isolations of virus from the these three species, OCK virus also has been isolated on three occasions from *Aedes cinereus* Meigen (Francy et al. 1989) and once from a pool of *Aedes* mosquitoes containing principally *Ae. communis* (De Geer) (Lvov et al. 1984). Because many *Aedes* species feed primarily on mammals but also will feed on birds (Service 1971), they may be the link between the enzootic *Culex-Culiseta*–bird cycle and human infections. To examine the potential of *Aedes* mosquitoes to serve as this link, we exposed field-collected *Ae. cinereus*, *Ae. communis*, and *Ae. excrucians* (Walker) mosquitoes to OCK virus by allowing them to feed on a viremic chick and examining them for infection, viral dissemination to the hemocoel, and transmission ability.

Materials and Methods

Mosquitoes. Adult female mosquitoes were collected in the vicinity of Sundsvall (about 380 km north of Stockholm), Sweden, during the latter half of July 1988 by three collection methods. These included quail-baited or dry ice-baited CDC miniature light traps and the aspiration of mosquitoes as they came to human bait. After capture, mosquitoes were provided apple slices as a carbohydrate source and transported to the National Bacteriological Laboratory in Stockholm, where they were held at 20°C until they were exposed to virus.

Virus and Virus Assay Procedures. The 84M140 strain of OCK virus, isolated from a pool of *Cs. morsitans* collected near Edsbyn, Sweden, in 1984 (Francy et al. 1989), was passed once in Vero cell culture before it was used in this study.

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[1] Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md. 21701.
[2] Department of Virology, National Bacteriological Laboratory, S-105 21 Stockholm, Sweden.
[3] Department of Virology, Karolinska Institute, S-105 21 Stockholm, Sweden.
[4] Swedish Defence Research Establishment, FOA-5, Box 27322, S-102 54 Stockholm, Sweden.

Specimens were assayed for the presence of OCK virus by plaque assay on Vero cell monolayers (Francy et al. 1989).

Infection of Mosquitoes. Mosquitoes were allowed to feed on restrained chicks (2–3 d old) that had been inoculated subcutaneously with $10^{8.2}$ plaque-forming units (PFU) of OCK virus 1 or 2 d previously. Immediately following the feeding period, a 0.1-ml blood sample was removed from the jugular vein of each chick to determine its viremia. Engorged mosquitoes were identified and placed in cardboard containers with netting at one end. These cages were placed in an incubator maintained at 17°C (the average daily temperature during the transmission season in the enzootic area) and a 20:4 (L:D) photoperiod. Apples were provided as a carbohydrate source, and moist gauze was provided as an oviposition substrate 7 d later. At selected intervals, mosquitoes were allowed to refeed individually on restrained 2–3-d-old chicks. After a 2-h feeding attempt, mosquitoes were cold-anesthetized, and their legs and bodies were triturated separately in 1 ml of diluent (10% heat-inactivated fetal bovine serum in Hanks' balanced salt solution with Hepes buffer) and frozen at −70°C until they were assayed for virus. Thus, it was possible to classify each mosquito as either uninfected, infected but with its infection limited to the midgut, or infected with a disseminated infection (Turell et al. 1984).

All chickens exposed to mosquitoes for transmission attempts were bled about 24 h after mosquitoes were removed. The blood was assayed for OCK virus on Vero cell monolayers. The recovery of OCK virus from this blood was considered evidence of successful transmission. Infection, dissemination (i.e., the percentage of mosquitoes with virus in their legs), and transmission rates were calculated for each mosquito species.

Results

Viremia levels in chickens at the time of mosquito feeding ranged from $10^{5.3}$ to $10^{7.2}$ PFU/ml blood. Mosquitoes were sampled 14, 21, and 28 d following the infectious blood meal. Within these dose and holding time ranges, we did not observe any effect of dose or of time of extrinsic incubation on infection, dissemination, or transmission rates. All three species were highly susceptible to per oral exposure; at least 96% of the individuals of each species became infected (Table 1). Viral dissemination to the hemocoel also was efficient in each of the species, as evidenced by recovery of virus from the legs of all 61 of the *Ae. excrucians* tested and from 51% (24 of 47) and 75% (6 of 8) of the *Ae. cinereus* and *Ae. communis* tested, respectively (Table 1).

Refeeding rates were low; only six mosquitoes were observed to take a second blood meal. However, *Ae. cinereus* and *Ae. excrucians* successfully transmitted virus by bite to chicks (Table 1). In addition to these six refeeding mosquitoes, two *Ae. excrucians* transmitted virus without visibly ingesting blood.

Table 1. Susceptibility of *Aedes cinereus*, *Ae. communis*, and *Ae. excrucians* to Ockelbo virus

Species	No. tested	No. (%) infected	No. (%) disseminated[a]	Transmission rate[b] (%)
Ae. cinereus	47	45 (96)	24 (51)	1/2 (50)
Ae. communis	8	8 (100)	6 (75)	NA
Ae. excrucians	61	61 (100)	61 (100)	2/4 (50)

[a] Percentage of all mosquitoes with virus in their legs.
[b] Number of refeeding mosquitoes transmitting/number refeeding (percentage transmitting); NA, none refeeding.

Discussion

This is the first demonstration of the ability of an *Aedes* mosquito to transmit OCK virus. All three mosquito species tested were highly susceptible to oral infection, and both species (in which individuals refed) transmitted virus by bite. Although the natural enzootic cycle for this virus appears to be between birds and either *Culex* or *Culiseta* mosquitoes (Francy et al. 1989, Lundström et al. in press), the mosquitoes implicated in Sweden (*Cx. pipiens*, *Cx. torrentium*, and *Cs. morsitans*) are primarily avian feeders which may not feed on man frequently (Service 1971, Jaenson & Niklasson 1986). In contrast, all three of the *Aedes* species tested in our study obtain most of their blood meals from mammals (including man [Jaenson & Niklasson 1986]), but they also will seek avian hosts as evidenced by their capture in quail-baited traps (data not shown) and presence of avian blood in field-collected, engorged specimens (Service 1971, Jaenson & Niklasson 1986). Although OCK virus has not been recovered from field-collected *Ae. excrucians*, only small numbers of that species have been tested. Ockelbo virus has been recovered from field-collected *Ae. cinereus* (Francy et al. 1989) and from a pool of *Aedes* mosquitoes consisting primarily of *Ae. communis* (Lvov et al. 1984). All three of these *Aedes* species are active and bite man during the latter half of July and August (Jaenson et al. 1986), the time of year when human infections of OCK virus occur (Espmark & Niklasson 1984). Although *Culex* or *Culiseta* species have been implicated as the principal enzootic vectors of most alphaviruses, *Aedes* species (such as *Ae. sollicitans* (Walker)) carrying eastern equine encephalomyelitis virus (Crans et al. 1986) and *Ae. melanimon* Dyar carrying western equine encephalomyelitis virus (Hardy 1987) have been incriminated in the transmission of these viruses to man and other mammals. Thus, *Aedes* mosquitoes should be considered a potential link between the enzootic *Culex-Culiseta*–bird cycle and human infections because they occur and feed on man during periods of OCK virus transmission, they are

susceptible to OCK viral infection, and they can transmit this virus by bite.

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